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# Efficacy of Hexaflumuron against the Fungus-Growing Termite *Pseudacanthotermes spiniger* (Sjöstedt) (Isoptera, Macrotermitinae)

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**Abstract:** The efficacy of hexaflumuron, a benzophenylurea insecticide, has been studied for the first time on a fungus-growing termite (*Pseudacanthotermes spiniger* Sjöstedt, Macrotermitinae). Results show that hexaflumuron could be useful in treating infestations of such pest species, which are of great economic importance in many tropical and equatorial countries. Foraging workers harvested and introduced treated food into the nest and subsequently contaminated the brood by trophallaxis. Hexaflumuron showed potent larvicidal activity. The compound did not appear to be rapidly degraded by the digestive enzymes of termite workers, nor by the symbiotic fungus *Termitomyces eurhizus* Heim growing on fungus combs. This chitin synthesis inhibitor did not act as a fungicide, since growth of the mycelium of this fungus was not inhibited *in vitro*. © 1998 Society of Chemical Industry

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Key words: fungus-growing termite; hexaflumuron; slow-acting toxicant; chitin synthesis inhibitor; *Pseudacanthotermes spiniger*

## 1 INTRODUCTION

Hexaflumuron is a benzophenylurea insecticide which acts by disrupting chitin deposition in the insect cuticle, so that the moulting process is inhibited and post-embryonic development is stopped. Recent laboratory studies and field tests have shown the high activity of this molecule against subterranean termites *Reticulitermes* and *Coptotermes*.[1–3] These species are lower termites, on which hexaflumuron may act not only on larvae but also on workers as these forms may undergo many moults after their differentiation.[4]

In this study, the action of hexaflumuron has been investigated for the first time on a fungus-growing higher termite (*Pseudacanthotermes spiniger* Sjöstedt). The main biological characteristic of higher termites is their symbiosis with a basidiomycete fungus, *Termitomyces spp.* which predigests the food collected by the workers outside the nest. The reproductives, soldiers and larvae are fed by the workers with this predigested food. The fungus-growing termites are of major economic importance. They are the most common termites in Africa and a major component of the Asian and Oriental faunas. Most of these termites feed on dead material but some species feed on living plants causing severe damage to many plant species. These termites are major pests of crops, plantations and forestry and, to a lesser extent buildings.[5,6] They also endanger earthen dikes and dams in South-East Asia, by building large subterranean cavities inside these structures.

The aim of this study was first to analyse the insecticidal potential of hexaflumuron in higher termites

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whose workers do not moult after their differentiation except to develop into soldiers.[4] We have studied the fungus-growing termite *P. spiniger* which proliferates in sugar cane plantations in many countries of tropical and equatorial Africa. The effects of hexaflumuron were observed on laboratory colonies either after topical application to reproductives, or after supplying workers with hexaflumuron-treated food. Hexaflumuron was also investigated as a potential fungicide, since the fungus walls are known to contain chitin. For this purpose, the growth of the mycelium was studied *in vitro* on media treated with hexaflumuron.

## 2 MATERIALS AND METHODS

### 2.1 Biological material

*Pseudacanthotermes spiniger* is an African fungus-growing termite living in savannah and in forest. It builds a hypogeous clayish nest, 60–100 cm in diameter, made of small adjacent chambers containing fungus combs. The reproductive pair (king and queen) live in a special chamber with thickened walls. Once a year, workers build an epigeous swarming dome where alates gather before their dispersal flight. This earthen dome may reach 150 cm in height and 50 cm in diameter.

This species feeds on dead leaves and wood collected at night by workers above the ground. This food is incorporated into fungus combs on which grows the symbiotic fungus *Termitomyces eurhizus*.[4,7,8]

Colonies of *P. spiniger* were reared in the laboratory (Dijon) at 28°C, 80% RH and under a 12 : 12 h light : dark daily cycle. The colonies have been raised since their foundation from alates collected in Gabon in 1988. They now contain several tens of thousands of individuals and each year produce several thousand alates. New incipient colonies are founded each year from these alates.

### 2.2 Hexaflumuron-treatments

Hexaflumuron 1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea) was supplied by DowElanco Specialty (Indianapolis, USA) (Technical grade 98.7%).

Hexaflumuron was applied topically to reproductives from three-month-old colonies which contained on average 10 individuals (larvae plus the reproductive couple). Insects were treated with hexaflumuron dissolved in 2 µl acetone by direct application to the abdominal cuticle at doses of 0·1 µg, 1 µg and 10 µg per individual ($n = 12$ colonies for each dose). Pure acetone (2 µl) was used as a control. Three control colonies and three treated colonies at each dose were observed at the beginning of the experiment and 15, 30 and 60 days after treatment by direct counting of numbers of eggs and larvae. In addition, the mortality of reproductives was observed at day 60.

To treat wood, a solution of hexaflumuron in acetone was evenly distributed on the wood surface with a glass pipette. Wood treated with 1 µg of hexaflumuron per mg of dry weight was supplied at the soil surface of nests of six-month-old colonies containing 40 individuals on average. Supplied wood was weighed at the beginning and at the end of the experiment. The amount of ingested hexaflumuron was estimated from the quantity of consumed wood. It was assumed that the wood supplied (*Jugulans nigra*), which comprised small pieces $2 \times 1 \times 0{\cdot}5$ cm of partly degraded and porous sapwood, was entirely impregnated by hexaflumuron solution. Samples of different termite castes and fungus combs were then analysed by HPLC (high performance liquid chromatography, see Section 2.3) for the presence of hexaflumuron, daily for five days. A similar experiment was carried out with a two-year-old colony containing about 40 000 individuals. All individuals were collected and weighed at the end of the experiment, and the population estimated from the weight of an aliquot sample. In this case, samples of the neuter castes were analysed 1, 3, 7 and 10 days after being supplied with treated wood. Hexaflumuron content was measured in reproductives after 15 days, at which time the colony was sacrificed.

### 2.3 HPLC analysis

Fungus combs or termites were homogenised in acetonitrile (0·5 ml). After centrifugation (5000*g* for 10 min), the residues obtained after evaporation of the supernatant in a vacuum centrifuge (Speedvac) were dissolved in acetonitrile + water (1 + 1 by volume; 250 µl). Aliquot fractions of 100 µl were injected into a reverse-phase Merck RP 18 column (125 mm length, 4 mm diameter, 5 µm particles). Elution was performed using a linear gradient of acetonitrile in water from 55% to 100% acetonitrile in 25 min. Analysis was performed with a Waters 600 E gradient chromatograph equipped with an automatic Waters 717 injector and a Waters 966 photodiode array detector. Data recording and integration were performed with Waters Millenium Software on a Nec Power Mate 433 computer. Chromatographs were analysed at 254 nm wavelength. Under these conditions, the retention time of the hexaflumuron standard was about 11 min. A systematic vertification by Millenium software of the UV spectrum of all detected residues, and comparison with a standard hexaflumuron, confirmed the identification of hexaflumuron in the extracts. For HPLC quantification, the peak area of hexaflumuron from each extract was measured by Millenium software and compared with known amounts of reference compound.

**TABLE 1**
Effects of Topical Applications of Hexaflumuron to Reproductives in Three-Month-Old Colonies: Evolution of the Number of Eggs and Larvae (Mean ± SD) and Mortality of the Reproductives. ($n = 12$ Colonies for Each Dose of Hexaflumuron)

| | | | *Hexaflumuron applications* | | |
|---|---|---|---|---|---|
| *Day* | | *Controls* | 0·1 µg | 1 µg | 10 µg |
| 0 | Number of eggs | 17 (±7) | 20 (±7) | 19 (±5) | 16 (±7) |
| | Number of larvae | 10 (±5) | 9 (±8) | 11 (±5) | 12 (±9) |
| 15 | Number of eggs | 20 (±7) | 20 (±4) | 15 (±8) | 17 (±7) |
| | Number of larvae | 14 (±7) | 8 (±5) | 3 (±3) | 3 (±2) |
| 30 | Number of eggs | 22 (±5) | 15 (±11) | 16 (±8) | 13 (±10) |
| | Number of larvae | 11 (±8) | 2 (±2) | 0 | 0 |
| 60 | Number of eggs | 21 (±7) | 7 (±1) | 1 (±1) | 0 |
| | Number of larvae | 14 (±10) | 0 | 0 | 0 |
| 60 | Reproductives' mortality (%) | 42 | 50 | 58 | 92 |

### 2.4 In-vitro cultures of *Termitomyces eurhizus*

The *T. eurhizus* mycelium was obtained from conidia collected on combs in a mature colony and cultivated *in vitro* on a solid medium[9] at 29°C. Hexaflumuron was tested at concentrations of 10 µg, 100 µg and 1000 µg per ml of medium (two replicates for each dose). The growth of the mycelium was estimated by measuring the fungus area.

## 3 RESULTS

### 3.1 Effects of topical application of hexaflumuron on reproductives

Table 1 shows that hexaflumuron acted not only on treated reproductives but also on untreated brood. The action of hexaflumuron on reproductives was observed on egg-laying and mortality. Sixty days after the beginning of the experiment, the number of eggs was greatly reduced in all treated colonies. The mortality of reproductives was only slightly higher in colonies treated with lower doses of hexaflumuron (0·1 and 1 µg) than in controls, but it was twice as high in colonies treated with 10 µg hexaflumuron. As regards the larvae, a noticeable reduction in their number was observed by day 15, and no larvae remained alive after 60 days. Three dead larvae were observed in one colony by day 15 and two dead larvae in two colonies by day 30.

### 3.2 Transmission of hexaflumuron within termite colonies

Results summarised in Table 2 suggest how the contamination of larger colonies (six months old) by hexaflumuron takes place. Such colonies were still young

**TABLE 2**
Transmission of Hexaflumuron within Six-Month-Old Incipient Colonies

| *Days* | $HFq^a$ (µg) | $FC^a$ (*ng HF* $mg^{-1}$ *FC*) | $LW^a$ | $SW^a$ | $S^a$ | $L^a$ | $K^a$ | $Q^a$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 164 | +[b] | −[b] | − | − | − | − | − |
| 2 | 124 | 22 | + | − | − | − | − | − |
| 3 | 295 | 7 | + | + | − | − | − | − |
| 4 | 197 | 26 | + | + | − | − | − | − |
| 5 | 85 | 6 | + | + | + | + | − | + |

[a] Hfq: quantity of hexaflumuron ingested by foraging workers (estimated from the quantity of consumed wood). FC: fungus combs, HF: hexaflumuron, LW: large workers. SW: small workers. S: soldiers (only small soldiers were measured). L: larvae, K: king. Q: queen.
[b] The sign '+' means the presence of hexaflumuron in the sample, but in insufficient amounts to be measured (less than 1 ng $mg^{-1}$ of fungus comb or per individual). The sign '−' indicates the absence of hexaflumuron detection in the sample.

**TABLE 3**
Transmission of Hexaflumuron within a Two-Year-Old Colony comprising *c.* 40 000 individuals

| | | | | (*ng HF per individual*) | | | | |
|---|---|---|---|---|---|---|---|---|
| *Days* | *Hfq*[a] (*mg*) | *FC* (*ng HF mg*$^{-1}$ *FC*) | *LW* | *SW* | *S* | *L* | *K* | *Q* |
| 1 | nd | + | + | + | − | − | nd[b] | nd |
| 3 | nd | + | + | + | + | + | nd | nd |
| 7 | nd | + | + | + | + | + | nd | nd |
| 10 | nd | + | + | + | + | + | nd | nd |
| 15 | 113 | 59 | 155 | 1 | + | 3 | − | + |

[a] All abbreviations as Table 2.
[b] nd = not determined.

and composed of relatively few individuals, but workers were already differentiated into large and small workers. Large workers foraged and collected food on the ground. The results show that they were the first individuals to be contaminated. The harvested food was rapidly incorporated into the fungus combs which became contaminated with hexaflumuron 24 h after introduction of the treated food. Small workers generally ensure the grooming and the feeding of reproductives, soldiers and larvae with saliva and predigested food of fungus combs. Hexaflumuron was detected in small workers three days after the beginning of the experiment. At five days, all the castes except the king were positive for hexaflumuron. Amounts of hexaflumuron, however, were not high enough to allow precise quantitative measurement.

Table 3 shows the results obtained with a two year-old colony containing one reproductive pair and 40 000 neuter individuals. The transmission of hexaflumuron within the colony appeared to be more rapid. Large workers, small workers and fungus combs were contaminated by hexaflumuron within 24 h after they were supplied with treated food, and all neuter castes, workers, soldiers, and larvae were found to contain small quantities of hexaflumuron three days after the start of the experiment. The colony was sacrificed 15 days after the beginning of the experiment and the reproductive pair was analysed. Only the queen was found to be contaminated with hexaflumuron.

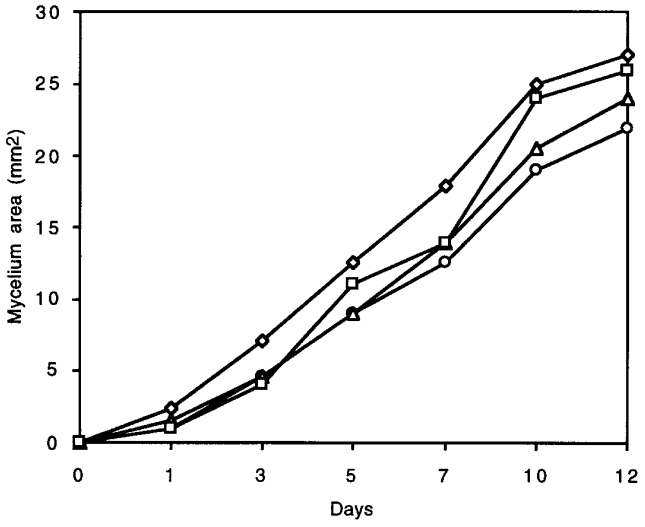


**Fig. 1.** In-vitro growth of *Termitomyces eurhizus* mycelium on media containing different concentrations of hexaflumuron (—○—) control (—◇—) 10 μg ml$^{-1}$ (—△—) 100 μg ml$^{-1}$ (—□—) 1000 μg ml$^{-1}$

### 3.3 Action of hexaflumuron on the growth of *Termitomyces eurizhus*

The growth of *T. eurhizus* mycelium cultivated on a control medium appeared linear during the 12 days of the experiment. The presence of hexaflumuron in the culture medium did not induce any visible effect on mycelial growth (Fig. 1).

## 4 DISCUSSION

Topical application of hexaflumuron to reproductives of the fungus-growing termite *Pseudacanthotermes spiniger* shows that this molecule is toxic to reproductives and also to brood which can be contaminated by trophallaxis. No larvae were present in colonies 60 days after an application of 100 ng of hexaflumuron per reproductive. This result could have arisen from an ovicidal effect of hexaflumuron as chitin synthesis inhibitors are known to induce the laying of non-viable eggs in several insect species.[10] It could also have arisen from a larvicidal effect of hexaflumuron, as strongly suggested by the presence of a few dead larvae in some colonies. Probably the larval death was due to failure to moult successfully, but the larvae were not well-preserved enough to verify this fact. As the larval population in control colonies was 10 individuals on average, the lethal dose can be estimated to have been less than

10 ng hexaflumuron per larva. Three nanograms of hexaflumuron were measured in the larvae of the large colony (two years old) 15 days after supplying treated food. Therefore, at active doses hexaflumuron is not rapidly toxic for the forager workers which can thus introduce the molecule into the colony, and so contaminate brood and reproductives. Hexaflumuron does not appear to be rapidly degraded either by the digestive enzymes of termite workers, or by the symbiotic fungus *Termitomyces eurhizus* as it can be certainly identified with HPLC by its retention time and its UV spectrum in different castes of termites and in fungus.

This study shows for the first time that hexaflumuron can be used against higher fungus-growing termites which are serious pests in many tropical and equatorial countries, not only of crops and plantations but also of buildings, dikes and dams. The authors have shown, with a highly sensitive HPLC method, that the reproductives and brood of populous colonies of such termites can rapidly be contaminated by hexaflumuron incorporated into food deposited on the ground, before the appearance of the first signs of insecticidal effects. Large workers collected contaminated food and small workers incorporated this material into fungus combs. Then they fed, by trophallaxis, the reproductives and the brood with saliva mixed with particles of fungus combs. The process was rapid, as fungus combs were contaminated within 24 h, and the female reproductives and larvae within five days, in young colonies. The absence of hexaflumuron in the male reproductives was probably due to less food being consumed by this caste.

From the results obtained with in-vitro cultures, hexaflumuron cannot be considered as a potential fungicide for fungus-growing termite control, since there was no observed effect on the growth of *Termitomyces* mycelium. This may seem surprising, as fungal walls contain chitin, and hexaflumuron is considered to be a chitin synthesis inhibitor. However, possibly due to differences in the metabolism of this polysaccharide in fungi and in insects, no benzophenylurea has been found to inhibit chitin synthesis in fungi.[10]

Our results thus allow us to envisage the use of the bait technique associated with the chitin synthesis inhibitor hexaflumuron to control pest species of fungus-growing termites, especially when these species attack buildings and earthen structures such as dikes or dams. However, these results must now be confirmed by field experiments in areas where alternative food sources are likely to be abundant. In such cases, the technique would probably need to be improved by using attractants.

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## REFERENCES

1. Su, N.-Y., Field evaluation of hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.*, **87** (1994) 389–97.
2. Su, N.-Y. & Scheffrahn, R., Laboratory evaluation of two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, as bait toxicants against Formosan and Eastern subterranean termites (Isoptera, Rhinotermitidae). *J. Econ. Entomol.*, **86** (1996) 1453–7.
3. Su, N. Y. & Scheffrahn, R., Fate of subterranean termite colonies (Isoptera) after bait applications—An update and review. *Sociobiology*, **27** (1996) 253–75.
4. Grassé, P.-P., *Termitologia*, 1. ed. Masson, Paris, 1982, 676 pp.
5. Logan, J. W. M., Cowie, R. H. & Wood, T. G., Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. *Bull. Entomol. Res.*, **80** (1990) 309–30.
6. Wood, T. G., The agricultural importance of termites in the tropics. *Agr. Zool. Rev.*, **7** (1996) 117–55.
7. Heim, R., Nouvelles études descriptives sur les agarics termitophiles d'Afrique tropicale. *Arch. Mus. Nat. Hist. Nat. Paris*, **18** (1942) 107–66.
8. Wood, T. G. & Thomas, J. R., The mutualistic association between Macrotermitinae and *Termitomyces.* In *Insect Fungus Interactions*, ed. N. Willing, N. M. Collins, P. M. Hammond & J. F. Weber. Academic. Press, London, 1989, pp. 69–92.
9. Thomas, R. J., Selective medium for isolation of *Termitomyces* from termite nests. *Trans. Biol. Mycol. Soc.*, **84** (1985) 519–26.
10. Muzzarelli, R., Chitin synthesis inhibitors: effects on insects and on non-target organisms. *Crit. Rev. Environ. Control*, **16** (1986) 141–6.